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(29)

Diabetes

Prevention of autoimmune diabetes mellitus in NOD mice by transgenic expression of soluble tumor necrosis factor receptor p55

The non-obese diabetic (NOD) mouse represents a relevant animal model of autoimmunity for insulin-dependent diabetes mellitus. The pathogenic role of tumor necrosis factor (TNF) in insulitis and β cell destruction observed in these mice remains controversial, since injections of TNF or of anti-TNF antibodies have been reported to exert protection or acceleration of diabetes, depending on the timing of administration. In this study, we demonstrate that, in contrast to the non-transgenic littermates, NOD mice with permanent neutralization of TNF by high blood levels of soluble TNF receptor p55-human FcIgG3-fusion molecules resulting from the expression of a transgene are protected from spontaneous diabetes. They are also protected from accelerated forms of disease caused by transfer of NOD spleen cells or cyclophosphamide injections. This protection is associated with a marked decrease in the severity and incidence of insulitis and in the expression of the adhesion molecules MAdCAM-1 and ICAM-1 on the venules of pancreatic islets. These data suggest a central role for TNF-α in the mediation of insulitis and of the subsequent destruction of insulin-secreting β-cells observed in NOD mice. They may be relevant to cell-mediated autoimmune diseases in general, in which treatment with soluble TNF receptors might be beneficial.

1 Introduction

The non-obese diabetic (NOD) mouse [1, 2] is a well established animal model for human insulin-dependent diabetes mellitus (IDDM). Besides environmental factors, at least ten different genetic loci are associated with disease development. The Idd1 gene which is linked to the MHC gene locus is essential for diabetes; all other diabetes susceptibility genes seem to contribute, but are not required for disease development [3, 4]. As in humans, IDDM in NOD mice is associated with an insulitis, characterized by a lymphocytic infiltration of the pancreatic islets. This may result in the progressive destruction of the insulin-secreting β cells, although the existence of insulitis in these mice is not sufficient by itself to lead to overt diabetes. Both CD4 and CD8 [5] T cells are involved in this process, which appears to be promoted by a T helper 1-type immune response [6]. TNF-α mRNA-expressing cells are found at very early stages of islet infiltration in spontaneous insulitis, as well as in the accelerated forms of IDDM resulting from the transfer into young syngenic recipients of spleen cells from diabetic NOD mice [7, 8]. This observation led to the creation of transgenic mice in a non-autoimmune background which constitutively and specifically release TNF in their pancreatic islets through a TNF transgene placed under the control of an insulin promoter (RIP-TNF transgenic mice). These transgenic mice develop a massive T cell insulitis [9, 10]; it was proposed that TNF-induced endothelial changes detected in venular islets, including expression of adhesion molecules for lymphocytes, were instrumental in the development of the insulitis [9]. These transgenic mice, however, do not develop overt diabetes, apparently because the islet-infiltrating T lymphocytes are not stimulated by local antigens. In spite of these observations, the issue of TNF involvement in insulitis and possibly diabetes of NOD mice is confused by discrepant observations made after the injection of TNF, or of anti-TNF antibodies, into newborn or adult NOD mice. Injection of TNF-α into newborn female NOD mice was found to accelerate disease development, whereas administration to adult animals was inhibitory [11]. Moreover, experiments involving neutralization of TNF-α with specific antibodies revealed both diabetes-protecting [11] and diabetes-promoting effects [12].

In an attempt to overcome limitations resulting from the use of neutralizing heterologous antibodies, we have generated NOD mice bearing a transgene directing the synthesis of soluble TNF receptor p55-human FcIgG3-fusion protein (sTNFR p55-hγ3) under the control of the α_1-antitrypsin promoter [13]. Mice bearing this transgene permanently express high blood levels of the fusion protein, which has been shown to neutralize large amounts of bioactive TNF-α; this results in a complete protection of this type of transgenic mice against a variety of *in vivo* pathological conditions mediated by TNF release [13]. The present report shows that NOD mice bearing this transgene fail to develop diabetes, either spontaneously or after

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Abbreviations: NOD mouse: Non-obese diabetic mouse **IDDM:** Insulin-dependent diabetes mellitus **sTNFR p55-hγ3:** Soluble TNFR p55, fused to the Fc part of human IgG3 **ICAM-1:** Intercellular adhesion molecule-1 **VCAM-1:** Vascular cell adhesion molecule-1 **MAdCAM-1:** Mucosal vascular addressin-1

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spleen, cell transfer or cyclophosphamide injections, two conditions which markedly enhance and accelerate the expression of diabetes in NOD mice. This protection was associated with a marked reduction in insulitis occurrence and severity, and absent or decreased expression of the adhesion molecules MAdCAM-1 and ICAM-1 on the endothelial cells of intraislet venules.

2 Materials and methods

2.1 Mice

NOD mice used in the present experiments originated either from Bomholtgard Breeding and Research Centre Ltd (Denmark) or from the animal facility of the Necker Hospital. The mice were kept and bred in the animal facility of the Medical School, University of Bern. A transgenic mouse B6D2F1 × B6D2F1, back-crossed twice with C57BL/6, expressing high levels of sTNFR p55-hγ3 under the control of the α_1-anti-trypsin promoter [13], was mated with female NOD mice. F1 males were subsequently back-crossed to NOD mice resulting in the back-cross 1 (BC1) generation. To generate BC2 animals, a BC1 female expressing sTNFR p55-hγ3 was mated with a male NOD mouse. For all other back-crosses, male transgenic animals were mated with female NOD mice. Transgenic mice were tested for homozygosity for H-2^{nod} by PCR amplification of the Hsp-68 microsatellite, located within the MHC class III region (primers for PCR kindly provided by H.-J. Garchon, Paris, France). In the present study, animals of the BC6, BC7 and BC8 generation were used. At this stage of back-crossing, the amount of non-NOD genetic background still present is estimated to be approximately 2 %. This includes about 20–25 centimorgan (cM) of genetic material selected with the transgene [14]. As transgene expression in heterozygous animals resulted in a consistently high expression of sTNFR p55-hγ3, all experiments were performed with animals heterozygous for the transgene, and with transgene-negative littermates serving as controls.

2.2 Transfer of diabetes

Adoptive cell transfer was performed as previously described [15]. Briefly, 2×10^7 splenocytes from overtly diabetic NOD mice were i.v. injected into irradiated (780 rad from a ^{137}cesium source) 8-week-old recipients.

2.3 Cyclophosphamide treatment

Female mice (15 weeks old) were injected twice at 2 weeks interval with 200 mg/kg cyclophosphamide (Alloxan, Asta).

2.4 Detection of sTNFR p55-hγ3 fusion protein

Mice were bled at 6 to 8 weeks of age and the concentrations of blood sTNFR p55-hγ3 fusion protein were evaluated by ELISA as previously described [13].

2.5 Determination of TNF-neutralizing capacity

TNF-neutralizing capacity of transgenic sera was assessed using a modified procedure described for TNF-α bioassays [16]. Briefly, 96-well plates (Costar), coated with 4×10^4 L929 cells per well were incubated overnight at 37 °C/5 % CO_2 with serial dilutions of mouse sera, preincubated with recombinant TNF-α (Innogenetics) in the presence of actinomycin D (4 μg/ml). Plates were subsequently stained with crystal violet and dried overnight. Methanol at 100 μl per well was added to solubilize the dye. TNF-α concentration was assessed by determining absorbance with an ELISA reader at 595 nm.

2.6 Tissue processing

Tissue specimens of a part of the pancreas were embedded in O.C.T. compound (Miles, Elkhart, IN), together with specimens from the spleen and small intestine as controls, and stored at − 70 °C. The remaining pancreatic tissue was immersed in 4 % paraformaldehyde in PBS, heated in a microwave oven as previously described [7] and embedded in paraffin by routine techniques.

2.7 Assessment of insulitis and diabetes

Mice were regularly tested (daily after adoptive cell transfer and cyclophosphamide treatment, weekly in untreated animals) for glucosuria with Tes-Tape (Eli Lilly, Indianapolis, IN) and were classified as diabetic after producing consistent Tes-Tape values of ≥ 2+. Insulitis was assessed by histology. For each animal, an average of 31 islets (range 15–62) on at least three non-serial hematoxylin and eosin-stained sections were analyzed by light microscopy. The severity of insulitis was assessed as periinsulitis (islet surrounded by few lymphocytes) and insulitis (lymphocytic infiltration into the interior of islets). In addition, the inflammatory infiltrate was evaluated and classified for each islet according to the following grading system: 0, intact islet; 1, area of mononuclear cell infiltration within an islet was < 25 %; 2, 25–50 %; 3, > 50 %; 4, final stage of insulitis characterized by small retracted islets with or without residual infiltrate [17].

2.8 Immunohistochemistry

Frozen sections containing pancreatic tissue specimens and mesenteric lymph nodes were double-stained with guinea pig anti-insulin antibody (Dako) and biotinylated monoclonal rat anti-MAdCAM-1 antibody (clone R3-3, kindly provided by B. Holzmann) or biotinylated monoclonal hamster anti-ICAM-1 antibody (clone 3E2, Pharmingen). Second-stage reagents were a rabbit anti-guinea pig antibody conjugated to peroxidase (Dako) and avidin conjugated to alkaline phosphatase (Dako), respectively.

2.9 *In situ* hybridization

Serial sections of paraffin-embedded pancreatic tissues were hybridized with ^{35}S-radiolabeled riboprobes for detection of the TNF-α gene as previously described [7].

2.10 Statistics

Evaluation of experimental data was performed using Student's *t*-test, and differences were considered to be significant with $p < 0.05$.

3 Results and discussion

In order to generate mice bearing the sTNFR p55-hγ3 transgene on the appropriate NOD background, a transgenic B6D2F2 mouse, back-crossed twice on a C57BL/6 background, expressing the transgene under the control of an α_1-anti-trypsin promoter [13], was mated with female NOD mice, and the resulting F1 transgenic males were back-crossed with NOD females as described in Sect. 2.1.

Activity of the α_1-anti-trypsin promoter in a transgenic mouse system has been reported already on day 12.5 of gestation [18]. Therefore, production of transgenic sTNFR p55-hγ3 is very likely to occur already *in utero*. This is further supported by the observation that neonates of the BC6 generation show high amounts (10–20 μg/ml) of sTNFR p55-hγ3 in the serum and reach still much higher adult levels of 290–1000 μg/ml at 3 weeks of age. As assessed in a L929 cytotoxicity bioassay, 1 ml of serum from newborn and adult mice neutralizes respectively about 1000 and 40 000 units (400 ng) of bioactive TNF.

In all experiments mice of back-cross generations 6, 7 and 8 (BC6-BC8), bearing one copy of the transgene, were compared to their transgene-negative littermates. As shown in Fig. 1A, the incidence of diabetes at 28 weeks in the non-transgenic mice was 36%, *i.e.* comparable to that of our conventional NOD mouse colony and to that of non-specific pathogen free colonies reported in the literature [19]. In contrast, none of 24 transgenic mice had developed diabetes. To assess whether transgenic expression of sTNFR p55-hγ3 protects NOD mice from onset of IDDM for an extended time period, six transgene-positive female animals were kept for more prolonged observation. Currently at the age of 42 weeks, none of these mice has become overtly diabetic yet. Matching this lack of diabetes in the transgenic animals, histological analysis of pancreata at two different time points (12 and 28 weeks of age) showed a decrease in the percentage of islets displaying insulitis in the transgenic mice compared to their non-transgenic littermates (Fig. 2).

To better quantitate the variations seen between different animals, pancreatic islets of the individual animals were also scored according to the system by Miller et al. [17]. This scoring system is based on the relative extent of mononuclear cell infiltration with 0 representing an unaffected and 4 representing a retracted islet completely lacking functional β-cells. At 12 weeks of age, sTNFR p55-hγ3-expressing NOD mice had an insulitis score of 0.2 ± 0.1

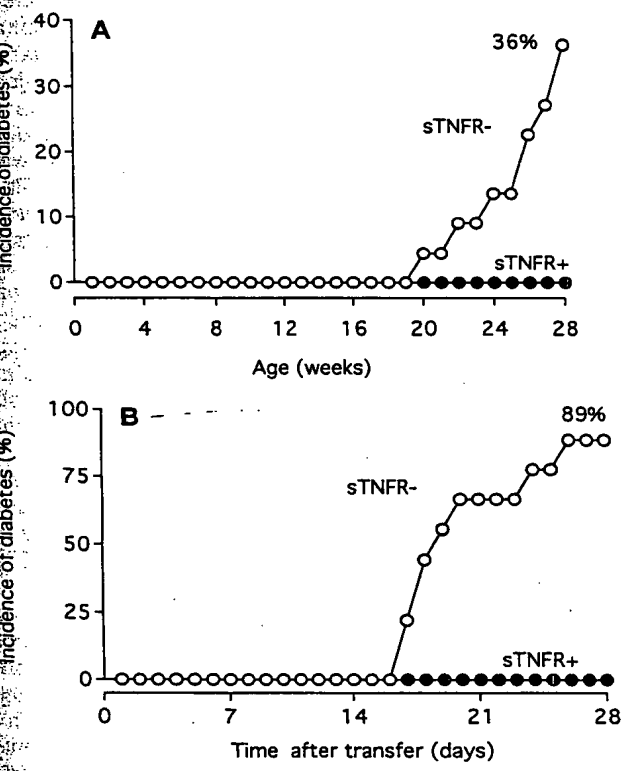

Figure 1. Incidence of diabetes in sTNFR p55-hγ3-expressing NOD mice (sTNFR+, filled circles) and in their transgene-negative littermates (sTNFR−, open circles). (A) Unmanipulated female mice (n = 24 for sTNFR+; n = 22 for sTNFR−). (B) After adoptive transfer of diabetogenic splenocytes (n = 15, 12 males and 3 females, for sTNFR+; n = 9, 6 males and 3 females, for sTNFR−).

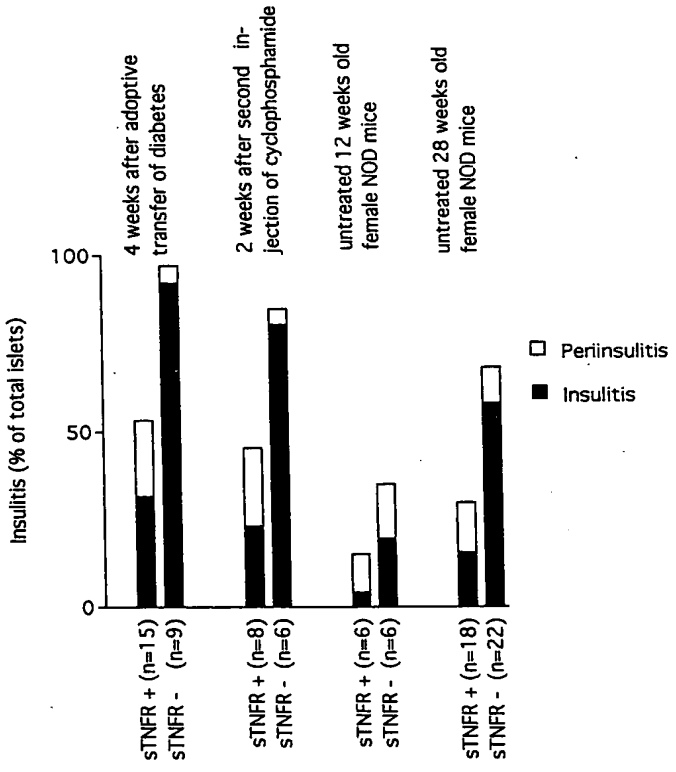

Figure 2. Severity of insulitis. Insulitis is shown as the percentage of infiltrated islets per total islets scored. For each time point, sTNFR p55-hγ3-expressing NOD mice (sTNFR+) and their transgene-negative littermates (sTNFR−) are indicated. For each animal at least three non-serial hematoxylin and eosin-stained sections were analyzed by light microscopy (15–62 islets per animal analyzed; n = numbers of animals examined).

(mean ± SEM; $n = 6$), and in non-transgenic littermates a score of 0.7 ± 0.2 (mean ± SEM; $n = 6$) was determined. This difference is statistically significant with $p < 0.05$. At 28 weeks of age, this difference was even more pronounced with a calculated insulitis score of 0.6 ± 0.2 (mean ± SEM; $n = 18$) in transgene-positive, and 2.1 ± 0.3 (mean ± SEM; $n = 22$) in transgene-negative littermates ($p < 0.001$). This protection is unlikely to be due to the co-transfer of IDDM resistance genes of C57BL/6 origin with the transgene. So far, the best characterized resistance genes are Idd3 and Idd10, on chromosome 3 and their cumulated effect, in heterozygous configuration, results in a reduction of disease incidence of maximally 25 % [20]. Furthermore, assuming that the transgene is on chromosome 3, there is little probability that the two resistance genes have been maintained together since they are 35 cM apart [4] whereas the estimated size of transferred DNA at BC6 is at most 25 cM [14]. Animals were screened by PCR amplification of the Hsp-68 microsatellite. This genetic marker is located within the MHC class III region at a short distance from the Idd1 locus, therefore, indicating with high probability homozygosity for Idd1. Further evidence for homozygosity of the Idd1 locus is provided by the fact that transgene-negative littermates of the BC6 generation show identical diabetes incidence as age- and sex-matched wild-type NOD mice in our colony. However, we cannot formally rule out the possibility that resistance to diabetes might have been conferred by some unidentified genes directly adjacent to and transmitted with the transgene. Other transgenic lines carrying the same transgene but with different integration sites would have offered the possibility of ascertaining whether the protective effect is associated only with the expression of the transgene. These transgenic lines exist [13], but have been selected to explore a correlation between the level of expression of the transgene and resistance to TNF effects; since they express lower levels of the fusion protein, which neutralize TNF incompletely or weakly, they could not be used for the present experiments.

To further confirm the protective effects of sTNFR p55-hγ3 against the development of IDDM, we transferred splenocytes from overtly diabetic mice of true NOD genetic background into irradiated adult BC6 recipients, bearing or not bearing the transgene. This type of adoptive transfer into 8-week-old male or female NOD mice has been shown to lead to a high incidence of diabetes within 4 weeks [15]. Whereas 89 % of the non-transgenic recipients (eight out of nine) developed IDDM 4 weeks after diabetes transfer, none of 15 transgenic recipients became diabetic during the same time period (Fig. 1B). Insulitis was present in almost all the examined islets of non-transgenic recipients (Fig. 2 and 3) and was more prominent than in 28-week-old mice without transfer (compare the corresponding columns in Fig. 2). Transgenic recipients showed a variable degree of insulitis ranging from almost absent in some mice to moderate in others (Fig. 3). Although the average incidence of altered islets in these transgenic mice was higher than in 28-week-old NOD transgenic mice without transfer, the degree of insulitis was nevertheless markedly lower than in 28-week-old non-transgenic NOD mice (compare the corresponding columns in Fig. 2). Four weeks after transfer of diabetes, the scoring of the insulitis according to Miller et al. [17] revealed in sTNFR p55-hγ3-expressing NOD mice an insulitis score of 1.3 ± 0.3 (mean ± SEM; $n = 15$) whereas in non-transgenic littermates an insulitis score of 3.5 ± 0.3 (mean ± SEM; $n = 9$) was observed ($p < 0.0001$).

Finally, 15-week-old female BC7 NOD mice were injected twice at 2 weeks interval with 200 mg/kg cyclophosphamide, a drug known to accelerate the occurrence of diabetes in NOD mice [21]. Two weeks after the second injection, three out of six non-transgenic mice developed overt diabetes, but none of the eight transgenic mice did. Again, protection from diabetes in transgenic mice was associated with a decreased level of insulitis (Fig. 2). The severity of insulitis is 0.9 ± 0.3 (mean ± SEM) for the eight transgene-positive animals and 2.8 ± 0.6 (mean

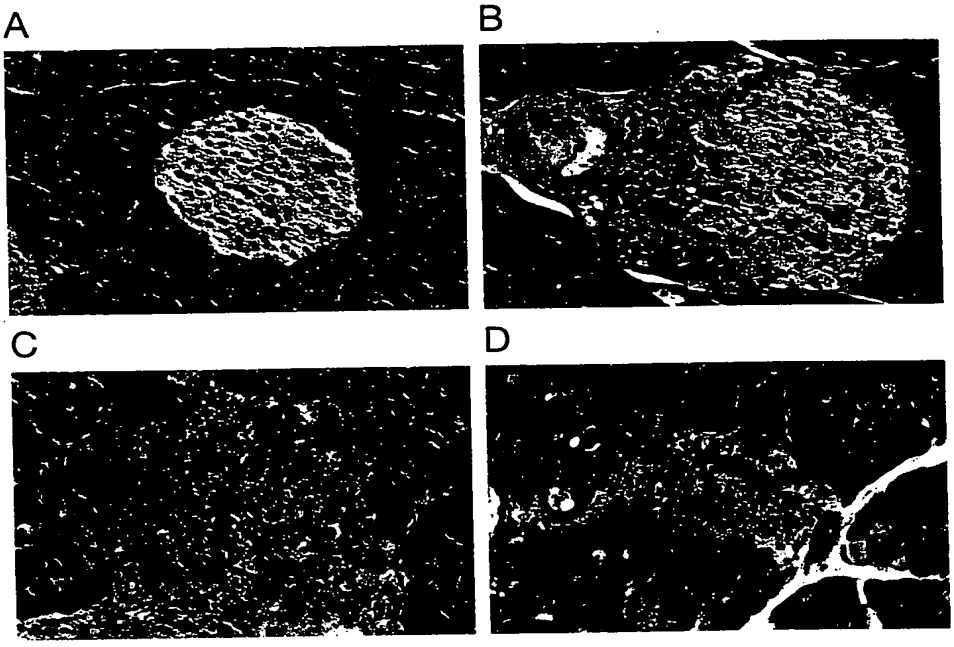

Figure 3. Insulitis 4 weeks after adoptive transfer of diabetes. Representative islets of Langerhans stained with hematoxylin and eosin. (A, B) Transgenic mice with no (A) and moderate (B) infiltration. (C, D) Transgene-negative littermates with heavily infiltrated (C) and atrophic (as a result of destruction) (D) islets. Magnification, × 40.

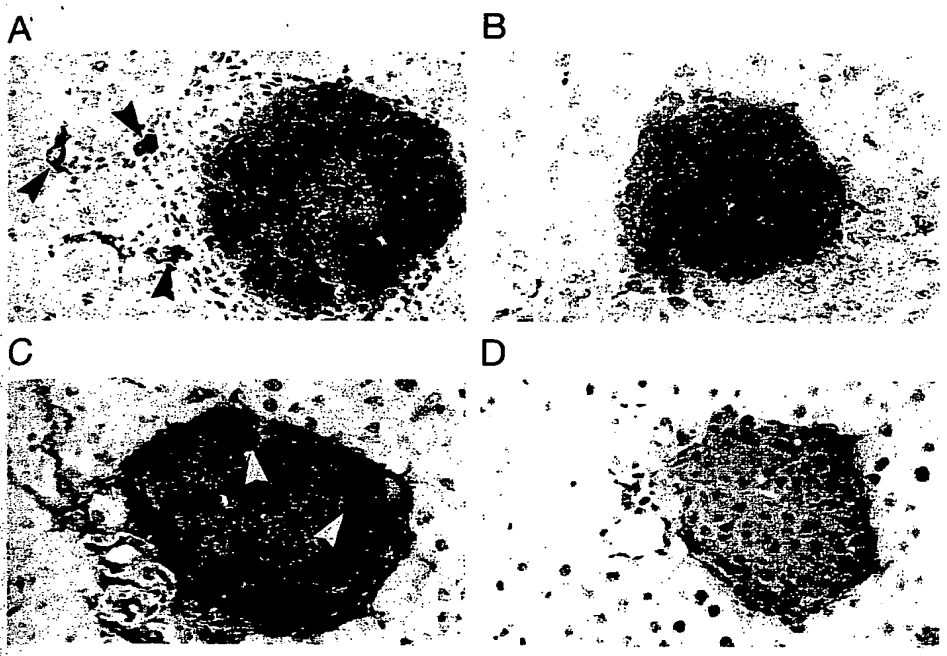

Figure 4. Representative islets of Langerhans, stained immunohistochemically for MAdCAM-1 (A, B) or ICAM-1 (C, D), respectively, and insulin (brown dye). Whereas in the islets of transgenic NOD mice (B, D), neither MAdCAM-1 nor ICAM-1 are detectable, the islets of their transgene-negative littermates express MAdCAM-1 on high endothelial venule (HEV)-like structures (A, black arrowheads), and ICAM-1 on HEV-like structures (C, white arrowheads) and other cells, possibly dendritic cells. Magnification, × 40.

± SEM) for the six transgene-negative littermates ($p < 0.01$) when assessed according to Miller et al. [17].

The protective effect on diabetes and insulitis of TNF neutralization by the sTNFR p55-hγ3 expressed in NOD transgenic mice may have been exerted at various levels. Since it has been reported that TNF, in combination with IL-1, has cytotoxic effects on pancreatic islets cells *in vitro*, the possibility may be considered that TNF released in the islets by the TNF mRNA-containing cells detected by *in situ* hybridization in the insulitis lesions [7, 8] (activated macrophages and/or activated T lymphocytes) may be directly cytotoxic for β cells. However, such a direct damaging effect of soluble TNF on islet β cells seems unlikely *in vivo*, since transgenic mice releasing TNF in pancreatic islets, while displaying a massive insulitis, never develop diabetes, as already mentioned [9, 10], even when injected with IL-1. Their β-cells are not decreased nor damaged when studied by immunochemistry and electron microscopy [9].

Membrane-bound TNF borne by infiltrating T lymphocytes may be instrumental in the process of T cell-mediated cytotoxicity which leads to diabetes by destroying the β-cells [22]. Membrane-bound TNF has indeed been shown to mediate lysis of various target cells, and it appears to be especially active on TNFR p75, whose stimulation may prime target cells for cytotoxic damage [23]. Concentrations of the fusion protein in the blood and tissues of transgenic mice are likely to be high enough to block the action of membrane-bound TNF-bearing cells. Nevertheless, in order to assess this possibility more directly, we used transfectants of the T cell hybridoma BY155.16 [24], which overexpress an uncleavable form of TNF-α and thus efficiently lyse TNF-α-sensitive L929 cells (Imboden, M. and Mueller, C., unpublished observations). Preincubation with serum from transgene-positive NOD mice, but not from transgene-negative littermates, already completely inhibited lysis of L929 target cells at a serum concentration of 3 %. This suggests that sTNFR p55-hγ3 may act in transgenic NOD mice by preventing or decreasing destruction of β cells through a mechanism of membrane-bound TNF-mediated cytotoxicity. Such an effect, however, would not easily explain the decrease in insulitis observed in the transgenic mice.

Alternatively, sTNFR p55-hγ3 could interfere with the spontaneous initiation of the autoimmune β cell response in NOD mice, systemically or locally, *i.e.* within the islets. Such an alternative would, however, not explain how diabetogenic effector T cells from wild-type NOD mice are prevented from causing disease when adoptively transferred into sTNFR p55-hγ3-transgenic recipients.

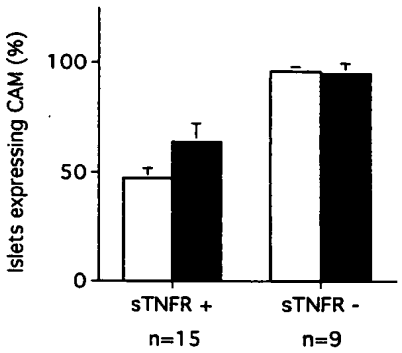

Figure 5. Percentage of islets of Langerhans expressing MAdCAM-1 (open bars) and ICAM-1 (solid bars), respectively, 4 weeks after adoptive transfer of diabetes in sTNFR p55-hγ3-expressing NOD mice (sTNFR+) and their transgene-negative littermates (sTNFR−) (± SEM). For each mouse, an average of 21 islets (range 10–50) on at least two non-serial pancreatic tissue sections were each examined for MAdCAM-1, and ICAM-1 expression, respectively (n = numbers of animals examined). Differences between sTNFR+ and sTNFR− mice were significant ($p < 0.0001$ for MAdCAM-1 and $p < 0.01$ for ICAM-1).

The last possibility, namely a decreased lymphocytic traffic in the islets, leading both to a decreased insulitis and to a decreased stimulation of autoreactive T lymphocytes by islet antigens appears to be a likely one, on the basis of the observations made with the RIP-TNF-transgenic mice [9]. In these mice, massive insulitis is accompanied by ultrastructural and autoradiographic changes of islet endothelial cells; it has been proposed that these endothelial alterations represent a direct effect of the local release of TNF on the endothelial cells, comparable to those observed on high endothelial venules of lymph nodes [9], in particular with respect to the expression of adhesion molecules such as MAdCAM-1 and ICAM-1 [25]. Moreover, besides these adhesion molecules, GlyCAM-1 is also strongly expressed in the islets of these transgenic mice (Herrera, P. and Vassalli, P., unpublished observations). Interestingly, both MAdCAM-1 and ICAM-1 have been found in infiltrated pancreatic islets of NOD mice [26, 27]. To test whether neutralization of TNF-α *in vivo* can diminish lymphocyte migration to the islets through down-regulation of endothelial adhesion molecules, pancreas sections of NOD mice after adoptive cell transfer were stained immunohistochemically for ICAM-1 and MAdCAM-1. Fig. 4 shows that, while ICAM-1 and MAdCAM-1 are observed on high endothelial venule-like structures on the islets of a non-transgenic mouse, cells expressing these molecules are either absent, or found in lower frequencies on islets of transgenic mice. Quantification of these observations (Fig. 5) appeared to parallel those made on histologic sections concerning the percentage of islets displaying insulitis in transgenic and non-transgenic mice shown in Fig. 2. This suggests that TNF-induced endothelial changes are correlated with the extent of insulitis, and thus, decreased up-regulation of these cell adhesion molecules may represent one mechanism of diabetes protection in sTNFR p55-hγ3-expressing NOD mice. Persistence of some endothelial alterations, associated with a decreased but nevertheless detectable insulitis in the transgenic mice might result from incomplete inhibition of locally produced TNF-α based on insufficient availability of the fusion protein in the islets for complete blocking of all TNF effects. Indeed, cells containing TNF-α mRNA detectable by *in situ* hybridization were still present in the islets of transgenic mice (Fig. 6). Alternatively, the synthesis and release of IL-1 in the islets could also be a mechanism for persisting insulitis in the transgenic mice, since IL-1 and TNF are known to induce comparable effects on endothelial cells *in vitro*.

Figure 6. In situ hybridization with a ^{35}S-labeled antisense probe for detection of TNF-α of an infiltrated pancreatic islet from an sTNFR p55-hγ3-expressing NOD mouse 4 weeks after adoptive transfer of diabetes. Magnification, × 60.

Once initiated, the process of insulitis may increase the chances that circulating lymphocytes, potentially autoreactive against islet antigens, penetrate into the islets and, as a result of antigenic stimulation, amplify the process. In this respect, it has been observed that the crossing of NOD mice with RIP-TNF-transgenic non-NOD mice which, as reported above, develop strong insulitis never accompanied by diabetes, leads to a high incidence of diabetes in the progeny, even at a low level of NOD back-crossing (Herrera, P. and Vassalli, P., unpublished observations). This observation which is unlikely to be related to the integration site of the transgene, since it was observed with the progeny of two founder mice, is also consistent with the facilitation by insulitis of the priming of a limited number of circulating autoreactive cells by their corresponding intra-islet antigens.

All these observations suggest that intra-islet TNF release in NOD mice, probably initiated by a limited T cell-mediated immune reaction, is instrumental in disease progression. Increase of local lymphocyte traffic enhances the probability of further recruiting and priming locally circulating autoreactive T lymphocytes, ultimately resulting in a sufficient accumulation of effector cells responsible for β cell destruction. This view on the action of TNF in autoimmune insulitis and IDDM is supported by several models of transgenic non-NOD mouse strains expressing TNF-α in their β cells, either alone or in combination with a second transgene directing the expression by β cells of foreign antigens or of the B7-1 co-stimulatory molecule [9, 10, 28–30]. These infiltration-promoting effects of TNF are not restricted to the pancreatic islets since neutralization of TNF by transgenic expression of sTNFR p55-hγ3 also efficiently inhibits infiltration to the salivary glands of NOD mice [31].

4 Concluding remarks

The present observations show that neutralization of TNF in the course of spontaneous or of accelerated IDDM of NOD mice prevents the occurrence of diabetes and decreases insulitis. Decrease of local lymphocytic traffic resulting from TNF-induced endothelial changes and expression of lymphocyte adhesion molecules probably play an essential role in this protection; an additional protective effect resulting from direct interference with a mechanism of membrane TNF-mediated cytotoxicity on β cells cannot be excluded. These data emphasize a central role of TNF-α during the process of autoimmune tissue destruction and also indicate beneficial effects of soluble TNF receptors in the treatment of organ-specific autoimmune diseases.

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